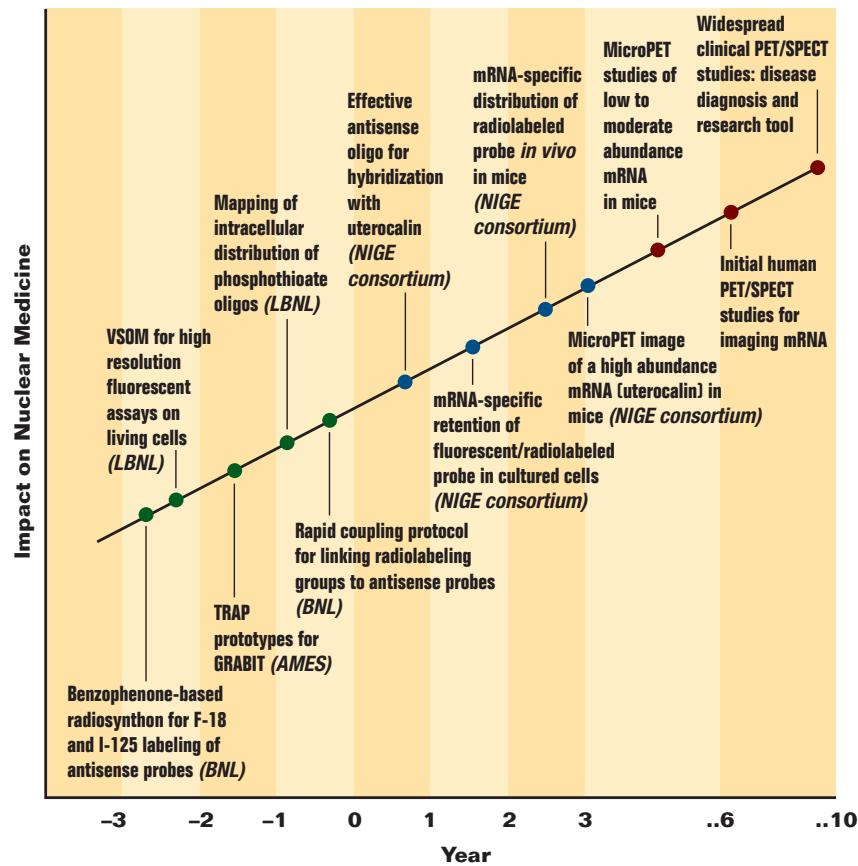


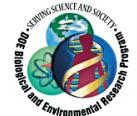
Collectively, these development stages represent the stepwise refinement of several novel imaging compounds and strategies that have been developed over the past three years at LBNL, BNL, and Ames. These three institutions will collaborate as a consortium that will focus on the same gene target (uterocalin) and employ the same antisense nucleotide sequence, the same model cell systems, and the same animal models. Compounds and imaging strategies will be moved through the various stages of development in a collaborative manner. Significant research synergism will maximize programmatic success.



## Timeline



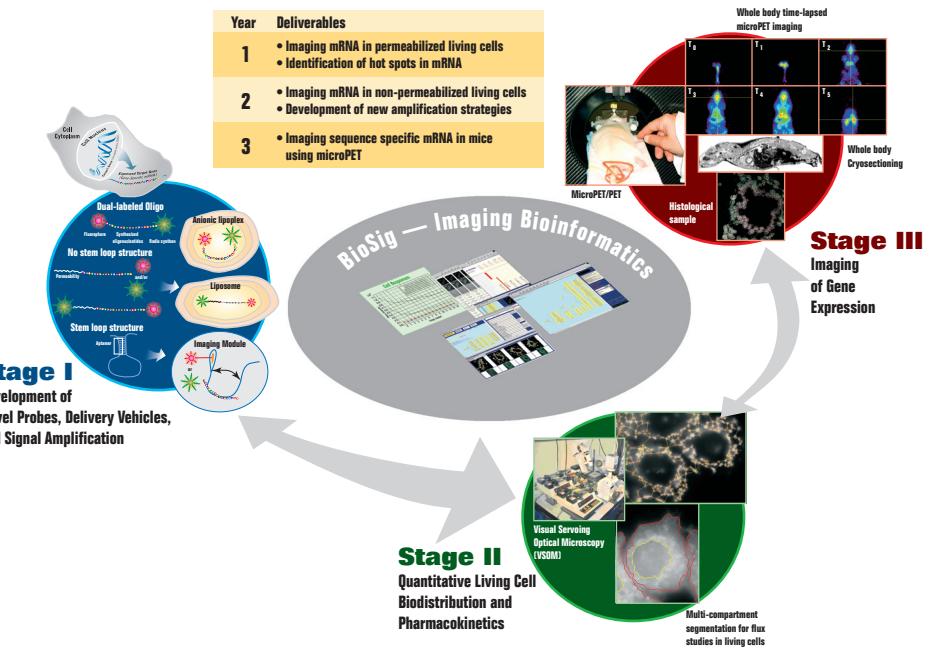
[vision.lbl.gov/NIGE](http://vision.lbl.gov/NIGE)



# NIGE

## Nuclear Imaging of Gene Expression

The primary objective of the Nuclear Imaging of Gene Expression (NIGE) consortium—Ames National Laboratory at Iowa State University (Ames), Lawrence Berkeley National Laboratory (LBNL), and Brookhaven National Laboratory (BNL)—is to overcome the technical challenges of directly imaging the expression of any gene in living cells, animals, and humans. The NIGE consortium will combine the expertise of these three institutions in an integrated three-step approach to developing gene-specific mRNA imaging agents. Synthetic antisense compounds and novel aptamer-based probes that can be detected using both fluorescent- and radio- labels will be used in conjunction with fluorescence microscope, single-photon emission computed tomography (SPECT), and positron emission tomography (PET) imaging modalities. This multi-institutional project will be supported by a unique imaging bioinformatics platform (BioSig) for distributing and sharing quantitative and annotated experimental results from each stage of the experimental pipeline among the respective investigators.



# In Vivo Gene Imaging

21st century medicine—innovative molecular imaging technologies from cell to human

## Identification and refinement of an optimal probe for gene imaging

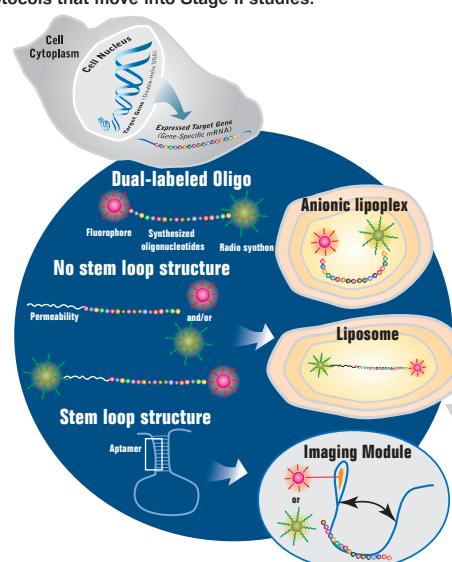
N I G E

# Nuclear Imaging of Gene Expression

## Stage I

### Development of Novel Probes, Delivery Vehicles, and Signal Amplification

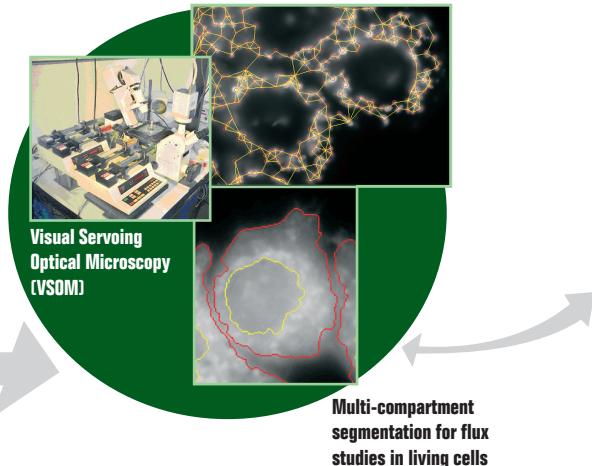
In **Stage I** (*probe chemistry and signal amplification*), novel synthetic antisense probes will be developed that are expected to be stable and non-toxic *in vivo*. Probe chemistry will focus on modified peptide nucleic acid (PNA) and antisense-aptamer designs that are compatible with (i) rapid and efficient protocols for radiolabeling the antisense probe or aptamer imaging module and (ii) incorporation of a near infrared (nIR) fluorophore that will improve probe detection in a spectral region free of biological autofluorescence. Probes will be characterized via MALDI-TOF mass spectrometry; amplification protocols will be characterized via BiAcore surface interaction techniques; and delivery vehicles containing probes will be characterized using techniques such as surface charge and particle size analysis. Only well-characterized probes, imaging modules, signal amplification protocols, and delivery vehicles will move into Stage II studies. Stage I results will also be used to prioritize the probes and protocols that move into Stage II studies.



## Stage II

### Quantitative Living Cell Biodistribution and Pharmacokinetics

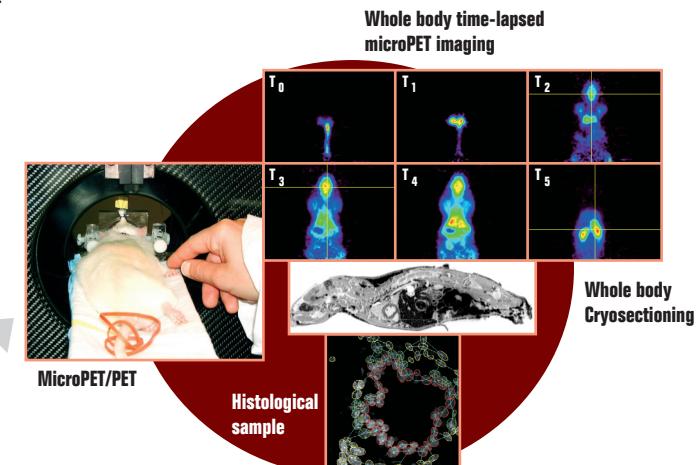
In **Stage II** (*living cell studies*), model cell systems (including custom-designed, sequence-specific transfectants) will be used to demonstrate sequence-specific mRNA imaging in living cells. Automated time-lapsed fluorescence microscopy coupled with embedded control of microperfusion pumps enable transient permeabilization (via reversible, streptolysin O-mediated membrane permeabilization) of living cells for direct delivery of the synthetic probes. Screening and validation will be augmented by novel computational methods for evaluating cellular flux data for iterative refinement of mRNA imaging protocols. Following successful demonstration of sequence-specific mRNA imaging in permeabilized living cells, studies will be further extended to probe delivery into nonpermeabilized cells through 3D fluorescence microscopy for a more accurate and complete representation. Successful development of assays for mRNA imaging in living cells will greatly assist functional probe design, signal amplification strategies, and delivery vehicles suitable for *in vivo* PET imaging of gene expression.



## Stage III

### MicroPET Imaging of Gene Expression

**Stage III** studies (*mouse biodistribution and pharmacokinetic studies*) will be performed using the prescreened and optimized probes and protocols produced in stages I and II. *Ex vivo* analyses will include tissue (whole body and specific organ) cryosections from injected animals that will be microscopically imaged to detect the accumulation or sequestration of labeled compounds in specific tissue compartments. Whole body cryosections will also be digitally imaged using a flatbed LiCor Odyssey nIR digital image scanner. Such studies will provide important data for the design of improved *in vitro* living cell assays that are capable of predicting *in vivo* biodistribution and pharmacokinetics. In experiments where radioactive versions of the antisense probes are used, autoradiographs of tissue sections will be obtained from appropriate flatbed digital imaging systems such as the PhosphorImager. In conjunction with *ex vivo* autoradiography and fluorescence imaging analysis, *in vivo* imaging using a Concorde microPET will be performed in order to determine probe or imaging module distribution in living mice.



## Core Competencies and Unique Expertise

Ames	Berkeley	Brookhaven	Ames	Berkeley	Ames	Berkeley	Brookhaven
• Innovative aptamer-based probe development	• Probe and delivery vehicle characterization	• Novel radiosynthons	• Model cell system	• Cellular pharmacokinetics	• Animal physiology	• Quantitative pharmacokinetics and biodistribution from PET images	• MicroPET studies of fluorine-18 labeled probes
• Cell biology and target gene	• Amplification strategy	• Rapid radiolabeling	• Cell and molecular biology	• Imaging bioinformatics	• Transgenic model	• Ex vivo functional probes	• Ex vivo autoradiography
• Identification of hot spot in mRNA		• PNA backbone chemistry	• Functional probe development	• Automation and biodistribution studies	• In vivo functional probes		• Whole body cryosectioning